UNIVERSITÉ DE LAUSANNE - FACULTÉ DE MÉDECINE
INSTITUT DE BIOCHIMIE
(Directeur : Professeur H. ISLIKER)

DETECTION OF RECURRENCE OF LARGE BOWEL CARCINOMA BY RADIOIMMUNOASSAY OF CIRCULATING CARCINOEMBRYONIC ANTIGEN (CEA)

THÈSE

présentée à la Faculté de Médecine de l'Université de Lausanne
pour l'obtention du grade de docteur en médecine

par

PHILIPPE JAEGER

Etudiant à la Faculté de Médecine de l'Université de Lausanne
Originaire d'Auboranges (Fribourg)

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Imprimerie Galley SA
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Faculté de Médecine

Le Conseil de la faculté de médecine de l'Université de Lausanne, après avoir pris connaissance des rapports favorables de MM. les professeurs H. Isliker et L. Freedman, et sans se prononcer sur les opinions du candidat, autorise l'impression de la thèse intitulée :

DETECTION OF RECURRENCE OF LARGE BOWEL CARCINOMA BY RADIOIMMUNOASSAY OF CIRCULATING CARCINOEMBRYONIC ANTIGEN (CEA)

présentée par Monsieur Philippe Jaeger, étudiant à la Faculté de médecine de Lausanne, pour l'obtention du grade de
DOCTEUR EN MÉDECINE

Lausanne, le 10 février 1975

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DETECTION OF RECURRENCE OF LARGE-BOWEL CARCINOMA BY RADIOIMMUNOASSAY OF CIRCULATING CARCINOEMBRYONIC ANTIGEN (C.E.A.)

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Summary The usefulness and limitations of the carcinoembryonic antigen (C.E.A.) radioimmunoassay for the evaluation of tumour resection and for the detection of tumour relapse were studied in patients with large-bowel carcinoma. The level of plasma-C.E.A. was determined before any treatment in a group of 101 patients with histologically proven adenocarcinoma of the colon and rectum. 71% of all patients and 63% of cases with localised tumour (Dukes A and B) had a preoperative C.E.A. value of 5 ng. per ml. or higher. This limit was reached by only 1 of 90 apparently healthy, non-smoking blood-donors. Among 45 patients for whom a complete tumour resection was reported, all patients except 5 showed a drop of C.E.A. to normal values after surgery. The 5 patients whose C.E.A. did not fall to below 5 ng. per ml. showed a subsequent rise in C.E.A. level and were all found later to have a tumour relapse. The results indicate that an incomplete drop of circulating C.E.A. level one month after surgery has a bad prognostic significance. 22 of these patients were followed up by repeated C.E.A. radioimmunoassay for several months after surgery. 8 showed a progressive increase in C.E.A. levels preceding clinical diagnosis of tumour relapse by two to ten months. 6 other patients showed a moderate increase in C.E.A. levels, suggesting a tumour relapse not yet clinically detectable. The remaining 8 patients showed no increase in C.E.A. level above 5 ng. per ml. and no clinical symptoms of re-

lapse. The results demonstrate that relapses of colon and rectum carcinoma can be detected by increased C.E.A. levels months before the appearance of any clinical evidence.

Introduction

CARCINOEMBRYONIC antigen (C.E.A.) was identified by Gold and Freedman in extracts of large-bowel carcinoma and in the digestive organs of human fetuses.[1] Circulating C.E.A. was first reported as detectable at concentrations above 2·5 ng. per ml. exclusively in the serum of patients with carcinoma of the colon or rectum.[2] However, more extensive studies utilising different radioimmunoassay methods have shown that raised C.E.A. levels are present in the blood of patients with several different types of cancer,[3–10] in non-neoplastic diseases (including cirrhosis of the liver,[3] ulcerative colitis,[13] regional ileitis,[13] pancreatitis,[13] chronic bronchitis,[6] and lung emphysema,[14] and in apparently healthy heavy smokers.[14,15] The demonstration of small amounts of C.E.A. in extracts of normal bowel mucosa,[16,17] normal liver,[18] and normal lung [19,20] could explain why inflammation in these organs might provoke the release of C.E.A. into the circulation. In addition, a moderate increase in C.E.A. levels has been found in the plasma or serum of apparently healthy African blood donors from Kenya,[21] whereas the concentrations in the blood of Europeans and Asians living in the same country were normal. These results, obtained independently at the Chester Beatty Research Institute in London and in our laboratory, could not be explained by the presence of any obvious endemic disease in the Africans.

It seems that the non-specificity of the C.E.A. test greatly limits its value for the early diagnosis of cancer, and most investigators agree that its major application lies in the follow-up after therapy of patients with diagnosed carcinoma.[13,22] However, despite the large number of C.E.A. tests already done all over the world, very few well-documented follow-up studies have yet been reported.[23,24] The purpose of this work is to investigate the usefulness of the C.E.A. radioimmunoassay for the evaluation of surgical resection of large-bowel carcinoma and for the surveillance of patients after operation. This study was performed in a two-year period, in collaboration with the surgical department of a general hospital. It illustrates the practical application of the C.E.A. test in a clinical setting.

Patients and Methods

Patients

1500 C.E.A. assays were performed on plasma from 800 patients with various malignant and non-malignant diseases. Of these patients, 101 had histologically proven adenocarcinoma of the colon and rectum, and will be presented in this report. Apart from the smoking history, which could not be obtained retrospectively in all cases, none of these patients had an associated factor likely to increase the circulating C.E.A. level. The smoking history was obtained in patients who were the subjects of the long-term follow-up study. The patients are separated according to Dukes' classification of tumour spread[25]

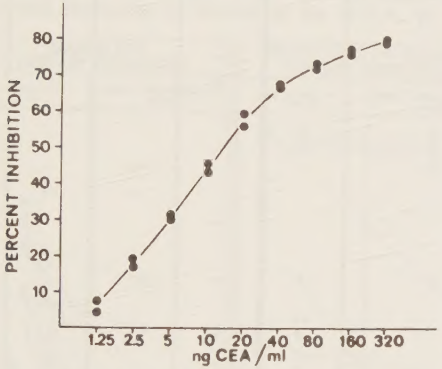

Fig. 1—Standard inhibition curve of the radioimmunoassay for C.E.A.

The ordinate shows the percentage of inhibition by increasing concentrations of non-labelled C.E.A. upon the binding of labelled C.E.A. to anti-C.E.A. antiserum. The abscissa shows the amount of purified C.E.A.[10] added in ng. per ml.

(fig. 2): (A) tumour limited to the bowel wall; (B) tumour spread through the serous coat without lymph-node invasion; (C) tumour spread to the local regional lymph-nodes; and (D) distant metastasis present.

The plasma-C.E.A. level was measured at least twice after surgery in 66 patients. A macroscopically complete tumour resection was reported for 45 of these patients (fig. 3, left): 12 cases of Dukes A, 18 of Dukes B, and 15 of Dukes C. In 19 patients, distant metastases were found or only a macroscopically partial tumour resection was possible (fig. 3, right): 1 case of Dukes B, 4 cases of Dukes C, and 14 cases of Dukes D. 2 patients were reported as having had a microscopically detectable tumour invasion in the edge of the sectioned bowel wall. The evolution of C.E.A. in these 2 cases (1 Dukes A and 1 Dukes B) will be described separately. In 6 patients a non-resectable cancer was found. In the 29 remaining

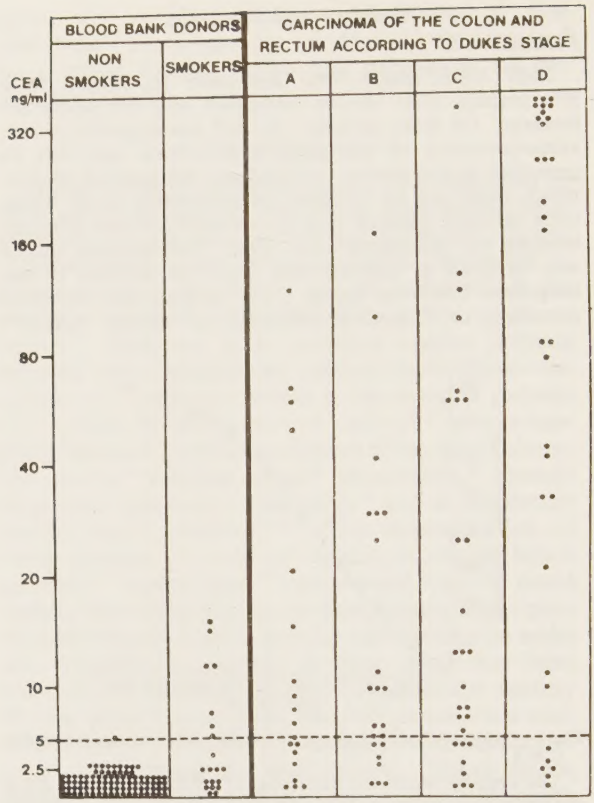

Fig. 2—Plasma-C.E.A. levels in 101 different patients with colon or rectum carcinoma before any treatment and in 110 apparently healthy blood-donors.

The patients are separated according to Dukes' classifications of tumour spread,[28] the blood-donors according to their smoking habits. Smokers = individuals smoking more than fifteen cigarettes a day for 5 years. Non-smokers also include individuals smoking less than fifteen cigarettes a day.

cases of the 101 with colon and rectum carcinoma, postoperative plasma samples were not available.

Among the cases with macroscopically complete tumour resection, 22 patients with no heavy smoking habits and a preoperative C.E.A. level higher than 3 ng. per ml. were followed up by repeated C.E.A. tests for a long time (figs. 4–6). The clinical course of the 2 representative patients who showed a tumour relapse preceded by a progressive increase in C.E.A. levels (fig. 4) will be described in case-reports.

Controls

110 apparently healthy blood-donors non-selected for

age and sex were taken as controls. 20 of them had been smoking more than fifteen cigarettes per day for more than five years, and 90 were either non-smokers or smoked less than fifteen a day.

Methods

The radioimmunoassay was performed using Gold's method [2] as modified by Mach et al.[8,9] The major modification was that duplicates of 2 ml. of plasma (10 ml. of blood was collected in tubes containing 33 mg. of dry E.D.T.A.-K_3) instead of 5 ml. of serum were extracted in 1 *M* perchloric acid. The sensitivity of the test is between 1 and 2 ng. per ml. (fig. 1). The reliability of this method was demonstrated in a study organised by the National Institutes of Health in the U.S.A. in which

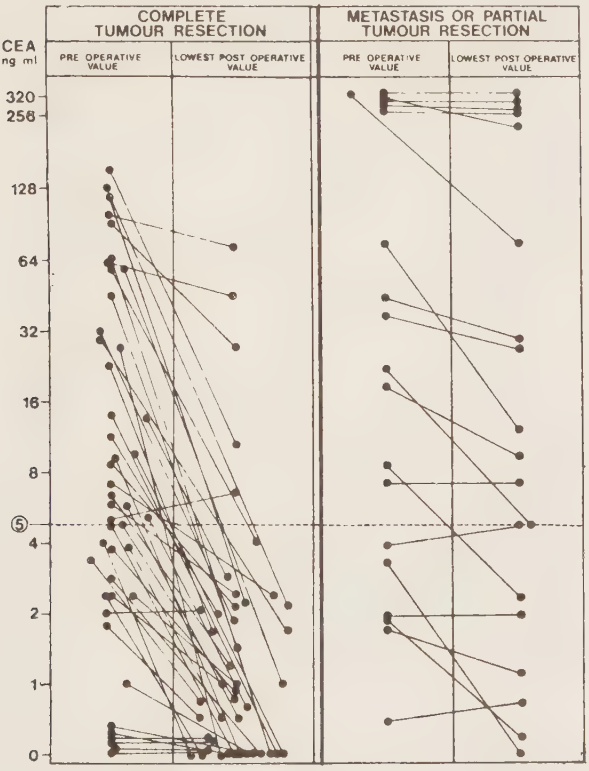

Fig. 3—Change in C.E.A. levels before and after surgery.

On the left, C.E.A. levels in 45 patients for whom a macroscopically complete tumour resection was reported. The 5 patients whose C.E.A. level did not fall below 5 ng. per ml. showed a subsequent rise in C.E.A. level and tumour relapse. On the right, C.E.A. levels in 19 patients with either distant metastases or a macroscopically partial tumour resection.

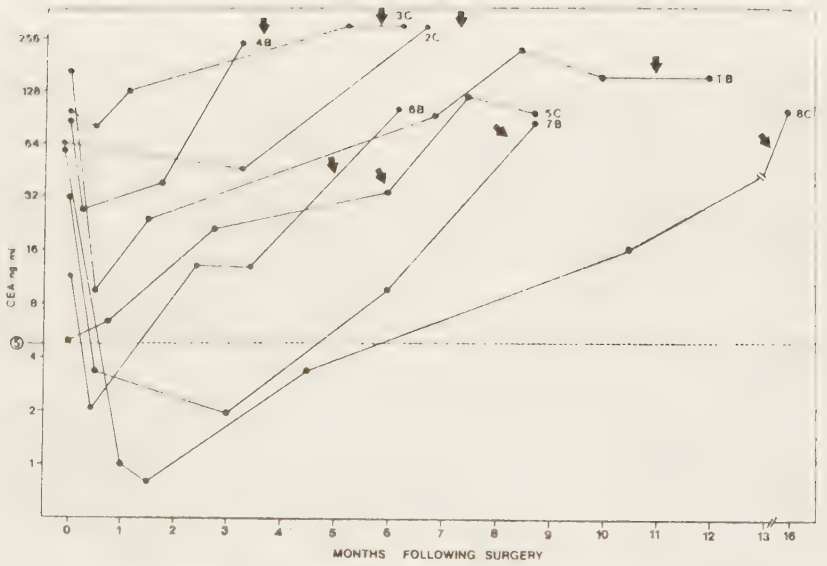

Fig. 4—Variations of C.E.A. level in 8 patients who showed a relapse of their carcinoma after what was considered as a macroscopically complete tumour resection.

The increased C.E.A. level was apparent between 2·5–10 months before clinical evidence of tumour relapse (arrows). For each patient after the final C.E.A. determination is the case number and the letter corresponding to the Dukes classification. For clinical course of patients 1 and 8 see case-reports.

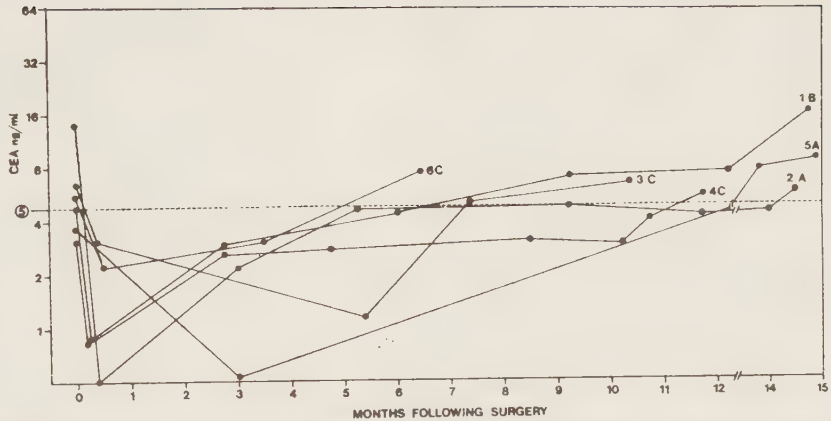

Fig. 5—Variation of C.E.A. level in 6 patients who, after a macroscopically complete tumour resection, showed an initial fall and a subsequent moderate increase in C.E.A. levels above 5 ng. per ml.

None of these patients had yet shown any clear evidence of tumour relapse. The letters correspond to the Dukes classification.

the C.E.A. levels of 94 blood specimens obtained from different patients were tested simultaneously by eleven different laboratories. Our results showed 82–90% correlation with those obtained by seven laboratories participating in the study—namely, those of Das, Go,[7] Gold,[2] Hansen,[14] Martin,[26] Todd,[25] and Zamcheck.[24] This correlation was obtained after transformation of the raw data by the formula $y=\log_{10}(x+0{\cdot}1)$, in which y= the transformed value and x the raw value. This transformation was necessary because the numerical C.E.A. results obtained by different methods of radioimmunoassay cannot be compared in absolute values. The C.E.A. results obtained by each individual laboratory should therefore always be interpreted in comparison with the values obtained in a series of normal controls. For instance, in healthy individuals the direct method originally described by Todd[25] and mostly used in Britain[6,11,27] will give values of up to 12·5 ng. per ml.; the method used by Stevens and Mackay[15] and the one used in our laboratory[8,9] will give values of up to 5 ng. per ml.; whereas the methods of Gold[2] and Hansen[14] will give values of up to 2·5 ng. per ml. To facilitate the comparison of results obtained by different methods a C.E.A. standard has been prepared by the National Institute for Biological Standards at Holly Hill, London, and is being tested by several laboratories. If found satisfactory, this standard will be submitted to W.H.O. to be used as the international standard.

Case-reports

A woman aged 64 had a well-differentiated adenocarcinoma of the splenic flexure of the colon (Dukes B). A round-shaped tumour measuring 2·5×2·5 cm. was removed, and the transverse colon was anastomosed to the skin. The C.E.A. level dropped from 160 ng. to 10 ng. per ml. two weeks after operation and later increased progressively up to 200 ng. per ml. Repeated alkaline phosphatases and transaminase determinations were normal. A hepatic scintigram seven months postoperatively showed no significant alteration. Eleven months after the first operation, the patient was reoperated on to re-establish the continuity of the colon. On this occasion, an inspection of the abdomen revealed no local tumour recurrence but a single liver metastasis of 2×2 cm. diameter diagnosed histologically as a differentiated adenocarcinoma. For ten months, the presence of this tumour relapse was suggested by the progressive rise of C.E.A. level while the patient was subjectively well and symptom-free.

A man aged 65 had a well-differentiated adenocarcinoma of the rectosigmoid (Dukes C). A large tumour mass (12×10 cm.) was macroscopically completely removed. The C.E.A. level fell from 60 to 1 ng. per ml. one month after surgery. An increase of C.E.A. level to 3·5 ng. per ml. was noted four and a half months after surgery and

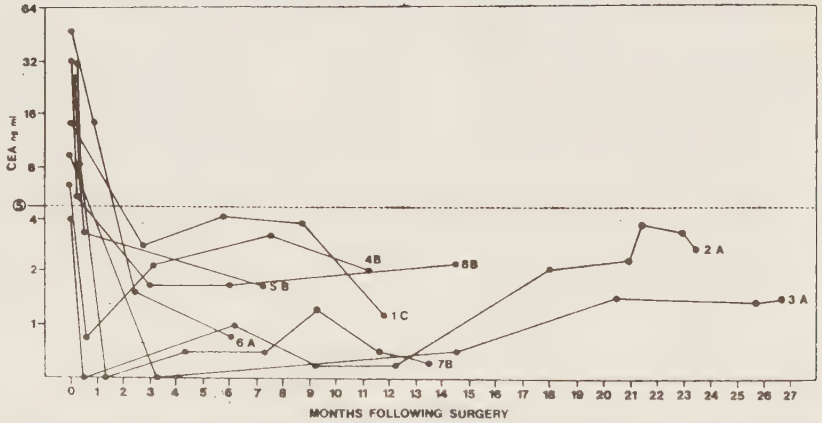

Fig. 6—Variation of C.E.A. level in 8 patients who, after macroscopically complete tumour resection, showed a fall of C.E.A. and whose C.E.A. value subsequently remained below 5 ng. per ml.

None of these patients showed any evidence of tumour relapse. The letters correspond to the Dukes classification.

to 16 ng. per ml. six months later. At this time, it was impossible to assess a local recurrence by clinical examination. Five months later, the C.E.A. rose to 100 ng. per ml., when a laparotomy was necessary to relieve a small-bowel obstruction due to pelvic recurrence of the carcinoma. The diagnosis of recurrence of the primary tumour was confirmed histologically.

Results

Correlation to Tumour Spread

The individual C.E.A. values of 101 patients with untreated adenocarcinoma of the colon and the rectum, and of 110 apparently healthy blood donors, are shown in fig. 2. The patients are separated according to the Dukes classification of tumour spread and the blood-donors according to their smoking habits. All 90 controls with no heavy smoking habits had C.E.A. levels below 5 ng. per ml. except 1. Therefore, 5 ng. per ml. will be considered as the cut-off limit in the description of the present results, even although, in general, C.E.A. values should always be considered quantitatively rather than interpreted as a positive or negative test.

Taking this arbitrary limit, raised C.E.A. levels are present in 11 out of 19 cases with Dukes A (58%), in 15 out of 22 cases with Dukes B (68%), in 20 out of 28 cases with Dukes C (71%), and in 26 out of 32 cases with Dukes D (81%). 71% of the 101 cases of large-bowel carcinoma had a C.E.A. level of 5 ng. per ml. or higher.

Among the 20 apparently healthy blood-donors with heavy smoking habits, 7 had C.E.A. values higher than the cut-off limit with a maximum at 16 ng. per ml. This accords with other reports.[14,15] The measurement of C.E.A. in these individuals was repeated after intervals of two to six months and the C.E.A. levels were stable. For some of the plasma samples, an alternative direct radioimmunoassay method as well as a different anti-C.E.A. antiserum were used; the increase in C.E.A. values remained within the same range. The comparison of the circulating C.E.A. levels in patients with carcinoma and in heavy smokers showed that roughly a third of patients with localised tumours and two-thirds of patients with metastases had C.E.A. levels above the highest value observed among heavy smokers (16 ng. per ml.).

Change of C.E.A. after Tumour Resection

The change of circulating C.E.A. level after surgery

in 45 patients for whom a complete tumour resection was reported is shown in fig. 3 (left). In all except 5 patients, the C.E.A. levels fell significantly to normal values or remained low after surgery. The 5 patients whose C.E.A. levels stayed above 5 ng. per ml. showed subsequent rises in circulating C.E.A. levels and tumour relapses. The evolution of C.E.A. in these cases is described later (see patients no. 1-5 in fig. 4). These 5 cases were 2 Dukes B and 3 Dukes C. The post-operative C.E.A. values, as well as the clinical evolution, suggest, however, that an undetectable distant metastasis was already present at operation. 4 other cases whose C.E.A. did not fall below 2·5 ng. per ml. were found to have Dukes C tumour. In all the other cases the C.E.A. level fell to or remained below 2·5 ng. per ml.

The change of circulating C.E.A. level after surgery in 19 patients in whom either distant metastases were found or only a macroscopically partial tumour resection was performed is shown in fig. 3 (right). In contrast to patients with resectable tumour, most patients in this group showed either little change in C.E.A. levels or a fall to values above 5 ng. per ml. after the resection of the primary tumour. However, 3 cases with low preoperative C.E.A levels (indicating the presence of a tumour secreting little C.E.A.) showed a fall of C.E.A. to undetectable levels after operation despite the presence of hepatic metastases. Based solely on results of C.E.A. assay, these 3 cases would have been misinterpreted as indicating complete tumour resection. A further 2 patients could not be included in fig. 3. They had a macroscopically complete tumour resection, but histological examination of resected tissue revealed that the tumour had infiltrated the bowel wall. In both patients, the C.E.A. level fell from preoperative values of 8 and 4 ng. per ml. to 1 ng. per ml. after operation, although the presence of residual tumour was demonstrated histologically.

Long-term Follow-up

22 patients with a reported complete tumour resection, who were not heavy smokers, were followed up by repeated C.E.A. assay to determine if a rise in C.E.A. level could help in the detection of tumour relapse. The evolution of circulating C.E.A in these cases is presented in figs. 4–6, with patients separated according to C.E.A. results and clinical course.

Fig. 4 shows the evolution of circulating C.E.A. level in 8 patients who had a relapse of their large-bowel carcinoma after what was considered to have been complete tumour resection. In each case inspection

of the liver showed no metastases. All these patients showed an increase of circulating C.E.A. level several weeks or months before the clinical diagnosis of tumour relapse (indicated by arrows). To demonstrate the usefulness and limitation of the C.E.A. radioimmunoassay in practical clinical conditions, 2 of these cases have been described above.

Fig. 5 shows the evolution of repeated tests in 6 different patients who had a moderate increase of C.E.A. level above 5 ng. per ml. without clinical symptoms of tumour relapse. Most patients of this group showed a similar evolution. Their C.E.A. dropped from moderately raised preoperative values to low level, and slowly increased to around the preoperative value six to twelve months after surgery. There is no sharp increase in C.E.A. values (as in fig. 4). Only 2 patients (nos. 2 and 4) showed suspicious clinical evidence of tumour relapse in the form of moderate weight loss and slight increase in alkaline phosphatases.

Fig. 6 shows the evolution of C.E.A. test in 8 other patients who had no increase of C.E.A. above 5 ng. per ml. None of these patients had any evidence of tumour relapse. Most patients showed a sharp drop of C.E.A. level after surgery except patient no. 1, who had a C.E.A. level which remained around 4 ng. per ml. for 9 months after surgery and then fell to 1 ng. per ml.

Discussion

Although the C.E.A. test has been in use for more than three years, it has been very difficult to make an objective evaluation of its usefulness in routine patient care.

We have shown that, in several patients, repeated C.E.A. tests gave valuable information about the presence of residual tumour or tumour relapse. Complete removal of a tumour was correlated with a fall to normal levels of C.E.A. Residual tumour and relapse were found to be related to persistently high levels of C.E.A. An increased C.E.A. level reflected a tumour relapse months before the appearance of any clinical evidence.

The use of the C.E.A. assay to evaluate the completeness of tumour resection should be critically considered. The absence of a fall of C.E.A. level to normal values one month after surgery was highly indicative of incomplete tumour resection (as illustrated by the five cases of fig. 3, left). However, the reverse is not true. A fall of C.E.A. to normal values, especially in cases with low preoperative levels, does not rule out the possibility of incomplete tumour resection (fig. 3,

right). The possibility of an associated pathology which can provoke the release of C.E.A. should also be considered in the interpretation of an incomplete drop of C.E.A. after surgery. In addition, the C.E.A. levels should not be judged too early after operation. Two or three weeks after operation, C.E.A. produced by the resected tumour can still be present in the circulation (cases 6–8 in fig. 6). Thus, the C.E.A. test should be repeated more than four weeks after surgery before drawing any conclusion.

The detection of tumour relapse by repeated C.E.A. assay is a more realistic possibility than detection of a primary tumour for four reasons:

(1) The lowest postoperative value can be used as a baseline to detect increased C.E.A. levels. Thus, even in the presence of an associated lesion such as chronic bronchitis or emphysema, an increase of C.E.A. can be considered as an indication of tumour relapse.

(2) Patients with large-bowel carcinoma, even after complete surgical resection, represent a high-risk population, justifying repeated C.E.A. tests.

(3) Regional recurrence or metastases of bowel carcinoma are often less symptomatic than primary tumours, making the C.E.A. relatively more important in the diagnosis.

(4) The selection of patients with tumours producing C.E.A. (as observed before operation) favours the earlier detection of recurrence. A relatively high proportion (63%) of patients with localised tumours (Dukes A and B) were found to have preoperative C.E.A. values of 5 ng. per ml. or higher (fig. 2). We consider that all these patients could be usefully followed up by repeated C.E.A. after surgery.

Since tumour recurrence after surgery has such a poor prognosis, what help will its earlier detection be to the patient? In this study we were only monitoring C.E.A. in relation to clinical symptoms of tumour relapse, and no therapeutic decisions were taken on the basis of the C.E.A. results. We consider, however, that, in the future, when two C.E.A. tests done at different times after tumour resection show a rise of C.E.A. level above previous values (and above values observed in normal controls), a second-look operation can be justified. A still resectable local tumour recurrence or a single hepatic metastasis may then be found and removed. In the case of non-resectable hepatic metastases, systemic or intra-arterial chemotherapy [29,30] might be initiated at an earlier stage than if the tumour were detected clinically. Another approach to the localisation of tumour relapse may be the injection of radiolabelled anti-

C.E.A. antibodies, as suggested by results obtained in hamsters [31] and "nude" mice [32] bearing heterografts of human colon carcinoma.

Only future studies can prove whether therapeutic decisions (e.g., surgery, chemotherapy, radiotherapy, or immunotherapy) based on C.E.A. results can lead to improved cures. The limited use of C.E.A. assay discussed here is much more modest than the original expectations of a test for early diagnosis of cancer. This is no reason, however, for neglecting the information that can be obtained, on some occasions, only by the measurement of circulating C.E.A. It is likely that, after an era of overoptimism followed by a reaction of disillusion, the C.E.A. test will find its precise application—namely, in the postoperative follow-up of selected cases of large-bowel carcinoma.

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Requests for reprints should be addressed to J.-P. M., Institut de Biochimie, 21 Bugnon, CH-1011 Lausanne, Switzerland.

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